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Normal chemotaxis in *Dictyostelium discoideum* cells with a depolarized plasma membrane potential

BERT VAN DUIJN^{1,2,*}, SAKE A. VOGELZANG^{1,2}, DIRK L. YPEY², LOEK G. VAN DER MOLEN¹ and PETER J. M. VAN HAASSTERT^{1,†}

¹Cell Biology and Genetics Unit, Zoological Laboratory, Leiden University, Kaiserstraat 63, NL-2311 GP Leiden, The Netherlands

²Department of Physiology and Physiological Physics, Leiden University, PO Box 9604, NL-2300 RC Leiden, The Netherlands

* Author for correspondence at the second address

† Present address: Laboratory of Biochemistry, Department of Chemistry, Groningen University, Nijenborgh 16, 9747 AG Groningen, The Netherlands

Summary

We examined a possible role for the plasma membrane potential in signal transduction during cyclic AMP-induced chemotaxis in the cellular slime mold *Dictyostelium discoideum*. Chemotaxis, cyclic GMP and cyclic AMP responses in cells with a depolarized membrane potential were measured. Cells can be completely depolarized by two different methods: (1) by treatment with azide; this probably causes inhibition of the electrogenic proton pump, which was shown earlier to regulate plasma membrane potential in *D. discoideum*. (2) By electroporation, which causes the formation of large non-ion-selective pores in the plasma membrane.

It was found that in depolarized cells the cyclic AMP-mediated cyclic AMP accumulation was inhibited. In contrast, chemotaxis to a cyclic AMP source was normal; the cyclic AMP-induced accumulation of cyclic GMP, which is known to mediate the chemotactic response, was also not affected. We conclude that membrane-potential-regulated processes, such as voltage-gated ion channels, do not play an essential role in chemotaxis in *D. discoideum*.

Key words: membrane potential, chemotaxis, H⁺-ATPase, ionfluxes, *Dictyostelium discoideum*.

Introduction

Chemotaxis plays an important role in the life cycle of the cellular slime mold *Dictyostelium discoideum*. During the vegetative stage the amoebae are chemotactic to folic acid and pterin (Pan *et al.* 1972, 1975), which are secreted by their food source, bacteria. When the food source is exhausted the amoebae aggregate to form a multicellular slug, which differentiates into a fruiting body. During cell aggregation chemotaxis is mediated by the chemo-attractant cyclic AMP, which is secreted by the cells (Bonner, 1947; Konijn *et al.* 1967). The binding of cyclic AMP to the surface cyclic AMP receptor elicits various intracellular responses, including a rapid but transient activation of adenylate cyclase and guanylate cyclase (see Janssens and Van Haastert, 1987; McRobbie, 1986; Newell *et al.* 1987). Experiments with *D. discoideum* 'streamer F' mutants show that the cyclic GMP response is implicated in the chemotactic response (see Ross and Newell, 1981; Janssens and Van Haastert, 1987; Newell *et al.* 1987). However, the intracellular cyclic AMP response is not involved in chemotaxis (e.g. see Newell *et al.* 1987; McRobbie, 1986).

Although many cellular responses are known to be associated with chemotaxis (Devreotes and Zigmond, 1988; Janssens and Van Haastert, 1987; McRobbie, 1986), it is still largely unknown how chemotaxis is regulated in *Dictyostelium*. Various experiments suggest a role for ions in cyclic AMP signal transduction and chemotaxis. A decrease in extracellular calcium concentration and an increase in extracellular potassium concentration upon addition of cyclic AMP to a suspension of cells have been reported (Aeckerle *et al.* 1985; Bumann *et al.* 1984, 1986; Malchow *et al.* 1982). Furthermore, cyclic AMP induces changes in the extracellular (Malchow *et al.* 1978) and intracellular pH (Aerts *et al.* 1987).

It may be expected that these changes in the distribution of ions over the plasma membrane cause changes in the plasma membrane potential. On the other hand, several aspects of signal transduction could depend on the plasma membrane potential. The relationship between membrane potential, membrane conductance changes and chemotactic movement of cells has been investigated in other cell types. In *Paramecium* spp. ciliary movement induced by chemo-repellents and chemo-attractants is regulated by membrane potential changes and ion fluxes

(see Kung and Saimi, 1982, 1985). In human neutrophils and macrophages chemotactic factor-induced ion fluxes (e.g. see Andersson *et al.* 1986; Simchowitch and Cragoe, 1986) and membrane potential changes (Gallin and Gallin, 1977) have been reported. In the non-cellular slime mold *Physarum polycephalum* membrane potential deflections are related to chemoreception and chemotaxis (Ueda *et al.* 1975, 1985).

We found earlier that the membrane potential of *D. discoideum* is mainly generated by an electrogenic proton pump (Van Duijn *et al.* 1988; Van Duijn and Vogelzang, 1989). This electrogenic proton pump might play a role in the cyclic AMP-induced ion fluxes, that were observed in *Dictyostelium*.

Two aspects of the relationship between ion fluxes, membrane potential and chemotaxis, can be considered. First, the membrane potential may be altered by chemoattractant-induced ion fluxes. Second, chemoattractant-induced alteration of the membrane potential may affect the function of membrane proteins (including ionic channels and pumps) that are involved in the chemotactic response. The second aspect was studied in *Dictyostelium* by measuring chemotaxis after artificially depolarizing the membrane potential with azide or by electroporation. The induction of non-ion selective pores in the plasma membrane by electroporation made it possible to study partly the role of ion fluxes in chemotaxis. The membrane potential depolarization by azide and electroporation was established using intracellular microelectrodes. We observed that cells with a depolarized membrane potential showed normal chemotaxis and conclude that membrane potential regulated processes do not appear to be essential in chemotaxis of *D. discoideum*.

Materials and methods

Materials

All chemicals were obtained from Sigma Chemical Co., St Louis, USA. The Na⁺-saline solution consisted of 40 mM-NaCl, 5 mM-KCl, 1 mM-CaCl₂ and 5 mM-Hepes-NaOH (pH 7.0). K⁺-saline solution consisted of 50 mM-KCl, 5 mM-NaCl, 1 mM-CaCl₂ and 5 mM-Hepes-KOH (pH 7.0). The K⁺-free saline solution was the Na⁺-saline solution without addition of KCl.

Culture conditions

Dictyostelium discoideum NC-4 (H) was grown in association with *Escherichia coli* 281 on solid medium containing 3.3 g peptone, 3.3 g glucose, 4.5 g KH₂PO₄, 1.4 g Na₂HPO₄·2H₂O and 15 g agar per liter. Vegetative cells are harvested with cold 10 mM-sodium/potassium phosphate buffer, pH 6.5 (PB), and washed free of bacteria by three washes and centrifugations at 150 g for 2 min.

Starved cells were obtained by incubation of cells on non-nutrient agar (1.5% agar in PB) at a density of 1.5×10^6 cells cm⁻² for 4.5 h at 22°C or 15 h at 6°C.

Membrane potential measurements

For membrane potential measurements the cells were deposited on glass coverslips with a thickness of 0.17 mm (about 5×10^4 cells cm⁻²). The glass coverslips were mounted on an open-bottom teflon culture dish, which was placed on the stage of an

inverted microscope (Ince *et al.* 1985), and cells were observed using 100× objective magnification with oil-immersion optics. During measurements the cells were bathed in a Na⁺-saline solution (see Materials), unless stated otherwise.

Membrane potential measurements were made with fine-tipped microelectrodes with wide-angle-tapers filled with 3 M-KCl. Electrode resistances were 57.0 Mohm (s.d.=18.2 Mohm, $n=119$), as measured in Na⁺-saline solution. The membrane potential recordings were stored on a digital oscilloscope using pre-trigger display (Nicolet 3091) and plotted afterwards, using an x-y plotter (Hewlett Packard 7035B).

The peak-value, E_p , of the fast potential transient observed within the first milliseconds upon microelectrode impalement of a *Dictyostelium* cell was considered to be the best available estimate of the membrane potential of *D. discoideum* (Ince *et al.* 1986; Van Duijn *et al.* 1988).

Chemotaxis

Cells were allowed to adhere to a glass coverslip mounted on a teflon culture dish. Chemotaxis towards a capillary (diameter 2–3 µm) filled with bath solution containing 10⁻⁵ M of the chemoattractant cyclic AMP was observed at 100× objective magnification. At $t=0$ s a short pressure pulse was applied to the capillary. At different time intervals photographs of the cells were taken. The distances between the tip of the capillary and the cells were measured from the photographs. By subtraction of these distances from the distance at $t=0$ the distance moved towards or away from the capillary by the different cells was calculated and plotted as a function of time.

Electroporation

Electroporation of *D. discoideum* cells was accomplished by applying two pulses of 7 kV cm⁻¹ with a RC-time of 210 µs, separated by a 2-s interval, to a suspension of 10⁸ cells ml⁻¹ (Van Haastert *et al.* 1989). For electrophysiological and chemotaxis experiments, 20 µl cell suspension was immediately placed in the teflon culture dish provided with a glass coverslip and filled with 1 ml Na⁺-saline solution. Adherence of cells to the glass coverslip occurred within 5 s after addition of the cell suspension.

Physiological and biochemical assays

Determination of ATP levels. Cells were treated with 0.1 or 1 mM-azide (NaN₃) for 0–30 min at 20°C at a density of 10⁸ cells ml⁻¹ in PB; 100 µl of the suspension were added to 100 µl perchloric acid (3.5%, v/v). Samples were neutralized with 50 µl KHCO₃ (50% saturated at 20°C) and analysed by high performance liquid chromatography (HPLC) on a LiChrosorp 10RP18 column that was eluted with 5 mM-tri-butylammonium phosphate, 100 mM-KH₂PO₄, pH 4.5, 10% methanol.

Cyclic AMP and cyclic GMP response. Cells (10⁸ cells ml⁻¹) were treated with azide or subjected to electroporation. To measure the cyclic AMP response, cells were stimulated with 5 µM-2'-deoxy-cyclic AMP and 5 mM-dithiothreitol, and to measure the cyclic GMP response the stimulus was 0.1 µM-cyclic AMP. At the times indicated in Fig. 4 (below) 100 µl samples were taken, and cyclic AMP and cyclic GMP levels were measured by isotope dilution assays (Van Haastert, 1984; Van Haastert and Van der Heijden, 1983).

Results

Depolarization of the membrane potential

Membrane potential measurements. The peak-value, E_p ,

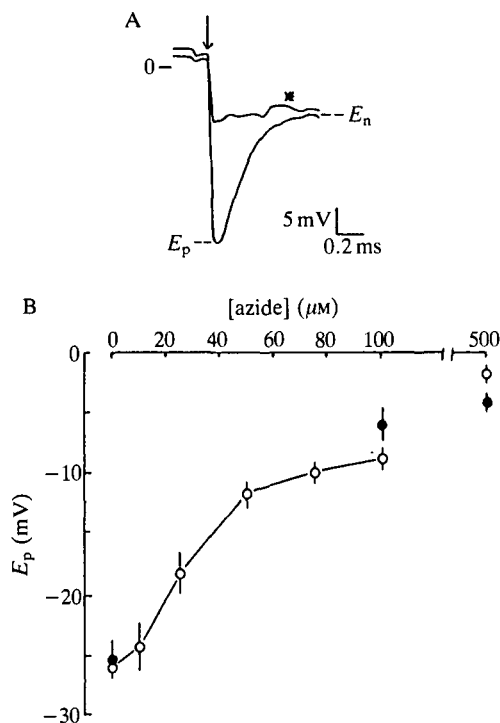


Fig. 1. Effect of azide on the membrane potential of *D. discoideum*. A. Potential transient recorded upon microelectrode impalement (moment of impalement indicated by arrow) of a cell bathed in Na^+ -saline solution. The peak-value of the potential transient, E_p , is a more reliable measure than the semi-stationary potential, E_n , for the true resting membrane potential prior to impalement. In cells preincubated with $100 \mu\text{M}$ -azide no potential transients were observed upon microelectrode penetration (recording indicated by *). B. Dependency of the mean peak-values of the potential transient, E_p , on the azide concentration as measured in vegetative cells bathed in Na^+ -saline solution (\circ , $n=353$, 26, 40, 35, 42, 40 and 19 for azide concentrations of 0 to $500 \mu\text{M}$, respectively). The E_p values measured in starved cells (\bullet , $n=68$; 24 and 11 for azide concentrations of 0 to $500 \mu\text{M}$, respectively) were not different from the E_p values in vegetative cells. Bars represent \pm S.E.

of the rapid potential transient observed upon impalement of a cell (cf. Fig. 1A; Ince *et al.* 1986; Van Duijn *et al.* 1988) was used as an indicator of the true membrane potential, E_m , existing before impalement. Penetration of a cell with a microelectrode introduces a shunt resistance that causes a rapid depolarization. When microelectrodes with sufficient small rise-times are used the rapid microelectrode-induced depolarization can just be recorded. Therefore, the value of E_p should be used as an indicator of E_m , rather than the depolarized value, E_n (Ince *et al.* 1986; Van Duijn *et al.* 1988). Fig. 1A shows a typical impalement transient as could be observed upon penetration of a *D. discoideum* cell.

Azide-induced depolarization. The effect of different azide concentrations on the value of E_p is shown in Fig. 1B. Azide depolarizes the membrane in a dose-dependent manner. At an azide concentration of $100 \mu\text{M}$ the membrane is almost completely depolarized and no peak potential transients could be observed (Fig. 1A).

These experiments were performed up to 30 min after

Table 1. Intracellular ATP concentrations after incubation of cells in different azide concentrations during different times

Azide concn (M)	Incubation time (min)	%ATP concentration (% of control)
0	—	100
10^{-4}	15	49
10^{-3}	15	21
10^{-4}	2	90
10^{-4}	30	27

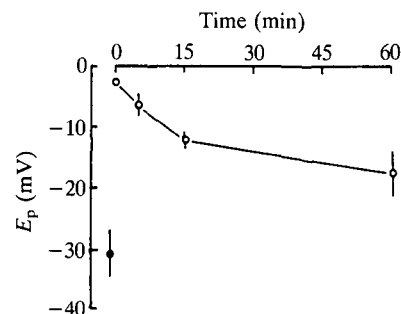


Fig. 2. Recovery from azide-induced depolarization. After measurement of the mean E_p value of cells bathed in K^+ -saline solution (\bullet , $n=24$), the cells were incubated in K^+ -saline solution with $100 \mu\text{M}$ -azide for 30 min. At $t=0$ the cells were washed free of azide and E_p values were measured as a function of time (\circ , total of 67 potential transient measurements). Bars represent \pm S.E.

addition of azide to the cells. Kinetic experiments revealed that the plasma membrane was depolarized before the first time point, i.e. within 3 min after addition of azide (data not shown). Azide had similar effects on the membrane potential in vegetative cells and aggregative cells (Fig. 1B).

Membrane depolarization can be caused by several processes, among which are membrane conductance changes, ion concentration changes, and electrogenic ion pump activity changes; we have tried to exclude some of these effects. Azide is a well-known blocker of the respiratory chain phosphorylation in the mitochondria. We measured the effect of azide treatment on cellular ATP levels by means of HPLC. Table 1 shows that intracellular ATP levels are only slightly reduced at 3 min after addition of azide concentrations that resulted in complete depolarization of the membrane potential. This indicates that energy depletion is probably not the main cause of the azide-induced membrane depolarization.

Changes of intracellular sodium and/or potassium ion concentrations may play a role in the azide-induced depolarization. We therefore measured E_p in cells bathed in solutions with different concentrations of K^+ and Na^+ . Cells bathed in K^+ -saline solution still possess a large negative membrane potential (Fig. 2). In this solution the Na^+ and K^+ concentrations are approximately equal to the normal intracellular concentrations (Aeckerle *et al.* 1985; Maeda, 1983; Marin and Rothman, 1980). Addition of $100 \mu\text{M}$ -azide to the K^+ -saline solution depolarized the cells (Fig. 2). Cells bathed in K^+ -free

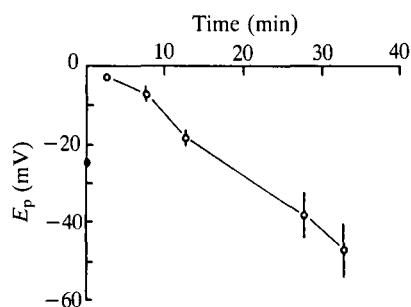


Fig. 3. E_p values measured in electroporated cells bathed in Na^+ -saline solution. Cells were electroporated at $t=0$. E_p value before electroporation is indicated by \bullet . Directly after electroporation E_p values were measured at different times (\circ , total of 77 potential transient measurements). Bars represent \pm S.E.

saline solution ($E_p = -20.5$ mV, S.D. = 6.1 mV, $n=15$) also showed depolarization upon addition of $100 \mu\text{M}$ -azide ($E_p = -5.1$ mV, S.D. = 2.7 mV, $n=18$). It thus appears that changes in sodium and potassium ion concentrations do not play a significant role in the azide-induced depolarization, which is supported by the observation that depolarization occurs immediately after azide addition.

These measurements indicate that the azide-induced depolarization is not caused by energy depletion, sodium/potassium ion concentration changes or membrane permeability changes for sodium and potassium ions. The membrane potential is largely generated by an electrogenic proton pump (Van Duijn *et al.* 1988; Van Duijn and Vogelzang, 1989), and it therefore seems likely that the azide-induced depolarization is due to inhibition of the proton pump activity by an unknown mechanism.

Recovery from azide-induced depolarization was measured in cells bathed in K^+ -saline solution after washing the cells free of azide (Fig. 2). Cells were incubated in $100 \mu\text{M}$ -azide for 30 min, and then the azide was removed. The time-constant of recovery was about 15 min, but recovery was never complete. Recovery from $100 \mu\text{M}$ -azide-induced depolarization in cells bathed in Na^+ -saline solution was not different from that of cells bathed in K^+ -saline solution (data not shown).

Electroporation-induced depolarization. High-voltage pulses can be used to make the plasma membrane of *Dictyostelium* permeable; under the conditions used large molecules such as inositol, but not ATP and inositol 1,4,5-trisphosphate, can cross the plasma membrane (Van Haastert *et al.* 1989). The introduction of large non-selective conductances should cause a depolarization of the membrane. Measurements in electroporated cells show that the membrane is completely depolarized immediately after electroporation (Fig. 3). All cells measured within 10 min after electroporation had an E_p value less negative than -10 mV. Recovery of the membrane potential is complete in about 40 min after electroporation. Recovered cells showed a much more negative E_p value as compared to control cells before electroporation (Fig. 3). This is a result of the increase in cell size

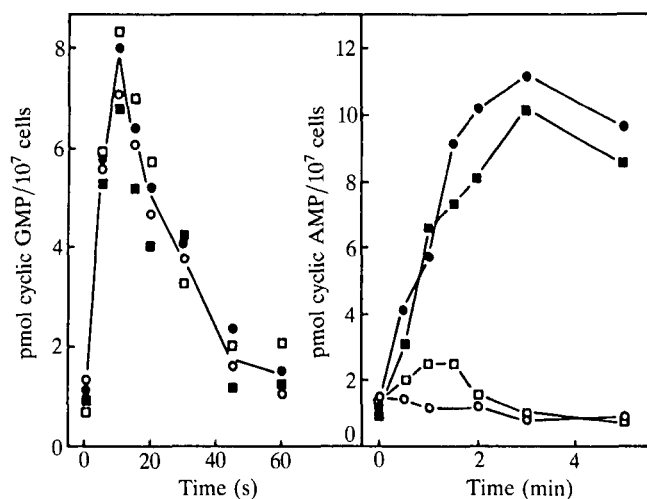


Fig. 4. Intracellular amount of cyclic GMP and cyclic AMP measured upon stimulation of starved cells with cyclic AMP. The graph shows the responses of control cells (\bullet), cells 30 s after electroporation (\circ), cells 30 min after electroporation (\blacksquare) and cells with addition of $100 \mu\text{M}$ -azide (\square).

due to electrofusion. In these enlarged cells E_p is closer to the true membrane potential (Van Duijn *et al.* 1988).

The experiments described above provide two different methods to achieve membrane potential depolarization: one causing a large increase in non-ion-selective membrane permeability (electroporation) and one causing no changes in membrane conductivity (azide). This offers the possibility of studying (1) the necessity of the membrane potential and (2) the role of ion fluxes in cell function.

Signal transduction

Chemoattractant-induced activation of guanylate cyclase is implicated in chemotaxis of *Dictyostelium*, but not the activation of adenylate cyclase (see Janssens and Van Haastert, 1987; Newell *et al.* 1987). The response of adenylate cyclase and guanylate cyclase upon stimulation with cyclic AMP was measured in suspensions of starved *D. discoideum* cells. Addition of $100 \mu\text{M}$ -azide before stimulation did not alter the cyclic GMP response (Fig. 4). However, the cyclic AMP response was absent in azide-treated cells (Fig. 4).

We also measured the cyclic AMP-mediated cyclic GMP and cyclic AMP response of electroporated cells at 30 s after the electroshock when cells still have a depolarized membrane potential. Fig. 4 shows that the cyclic GMP response is still present in electroporated cells. The cyclic AMP response, however, is absent in electroporated cells (Fig. 4). The cyclic AMP response is restored at 30 min after electroporation; at this moment the membrane potential is also restored.

Chemotaxis in depolarized cells

We next studied the influence of both azide and electroporation on chemotaxis towards a cyclic AMP source.

Fig. 5 shows the chemotactic response of cells bathed in Na^+ -saline solution. Control cells rapidly move to the microcapillary filled with cyclic AMP upon application of

a pressure pulse (Fig. 5A,B). Quantitative data are presented in Fig. 6. The chemotactic response of cells in $100\ \mu\text{M}$ -azide, which completely depolarized the membrane potential, is identical to the response of the control cells (Fig. 6). After more than 30 min in $100\ \mu\text{M}$ -azide chemotaxis was still unaffected (data not shown). Chemotaxis was also measured in electroporated cells. Fig. 5C–F demonstrates that these cells respond chemotactically towards the cyclic AMP source. The quantitative data (Fig. 6) reveal that chemotaxis was not significantly (Student's *t*-test, 95 % level) different from the response of control cells.

Discussion

In the present study chemotaxis and signal transduction were investigated in *D. discoideum* cells with depolarized membrane potentials. The experiments show that both azide and electroporation induce membrane depolariz-

ation in *D. discoideum* cells. The main result of this study is the presence of normal chemotaxis towards cyclic AMP in completely depolarized *D. discoideum* cells.

Membrane depolarization

Membrane depolarization can be achieved either by treatment with azide or by electroporation. Azide-induced depolarization does not depend on the K^+ or Na^+ concentration in the extracellular medium, which indicates that depolarization is not caused by azide-induced changes in sodium and/or potassium ion permeability and/or ionic gradients. Also in *Neurospora* azide does not affect membrane permeability (Slayman, 1965).

In *Neurospora* and yeast cells azide-induced depolarization is thought to be due to azide-induced ATP depletion. However, azide-induced membrane depolarization precedes azide-induced reduction of oxygen consumption in both *Neurospora* and yeast cells (Slay-

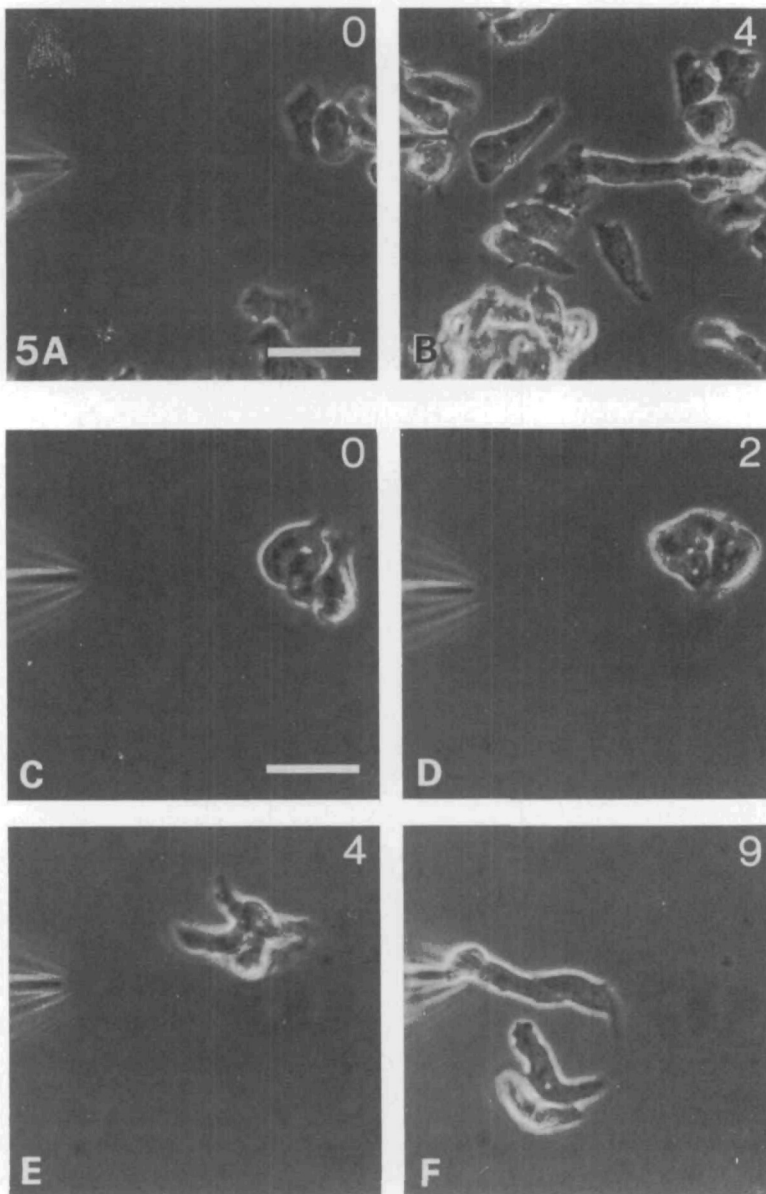


Fig. 5. Micrographs showing chemotaxis towards a capillary filled with $10^{-5}\ \text{M}$ -cyclic AMP in Na^+ -saline solution. Bar, $20\ \mu\text{m}$. Time is indicated in minutes. A–B. At $t=0$ min a short pressure pulse was applied to the capillary (A). At $t=4$ min many cells reached the tip of the capillary (B). C–F. Chemotaxis of electroporated starved cells, bathed in Na^+ -saline solution, towards the capillary. Cells were electroporated 75 s before $t=0$. At $t=0$ a short pressure pulse was applied to the capillary (C). At $t=9$ min cells reached the tip of the capillary (F).

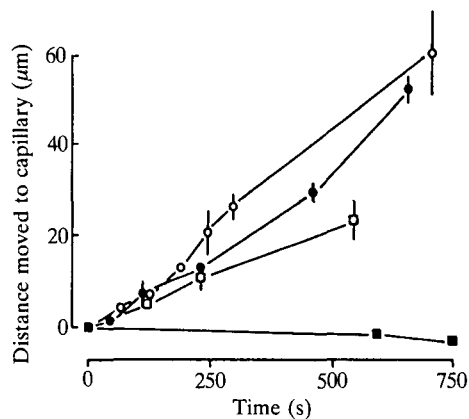


Fig. 6. Distance moved in time in the direction of the capillary by starved *D. discoideum* cells bathed in Na^+ -saline solution. At $t=0$ a short pressure pulse was applied to the capillary. Chemotaxis of cells towards a control capillary (■, mean of 31 cells) and a capillary filled with 10^{-5} M-cyclic AMP (●, mean of 36 cells). Chemotaxis towards the capillary filled with 10^{-5} M-cyclic AMP of starved cells in a solution with $100 \mu\text{M}$ -azide (○, mean of 20 cells) and electroporated starved cells (electroporation 75 s before $t=0$; □, mean of 16 cells). Bars represent \pm S.E.

man, 1965; Foulkes, 1956), indicating a more complex inhibition mechanism. In our experiments the azide-induced depolarization in *D. discoideum* cells seems not to be due to ATP depletion: intracellular ATP levels are not largely decreased at 3 min after addition of $100 \mu\text{M}$ -azide, a treatment that completely depolarized the membrane potential. Furthermore, normal cell movement is observed in azide-treated cells.

Several ion-dependent plasma membrane ATPases (ion pumps) have been identified biochemically in *Dictyostelium*. Some of these ATPases can be inhibited directly by azide (Blanco, 1982). However, the electrogenic proton pump, which mainly generates the membrane potential of *Dictyostelium* (Van Duijn and Vogelzang, 1989), was found to be insensitive to azide in biochemical assays (Pogge von Strandmann *et al.* 1984; Serrano *et al.* 1985). Possibly, the azide-induced depolarization is caused by indirect inhibition of the electrogenic proton pump, which is not due to ATP depletion. The high-affinity plasma membrane Ca^{2+} -ATPase in *Dictyostelium* described by Böhme and coworkers (1987) can be inhibited by azide *in vivo* but not *in vitro*. They suggest that azide might inhibit transport of high-energy phosphate compounds to the cell periphery (Böhme *et al.* 1987). Future research is required to elucidate the mechanism of azide-induced depolarization in more detail.

Electroporation also causes membrane depolarization. Immediately after electroporation all cells measured were completely depolarized. Electroporated cells in suspension show uptake of inositol up to 10 min after applying the high voltage (Van Haastert *et al.* 1989). Therefore, we conclude that the electroschock-induced depolarization is due to the introduction of non-ion-selective pores in the plasma membrane.

From the measurements we conclude that the azide-

treated and electroporated cells used in the chemotaxis and signal transduction experiments all have a completely depolarized membrane potential, and that electroporation will induce non-ion selective fluxes across the plasma membrane.

Chemotaxis and signal transduction

Many physiological responses, among which are ionic events, are associated with chemoreception and chemotaxis (see Devreotes and Zigmond, 1988). In different amoeboid cells chemoattractant-induced membrane potential changes have been reported (Gallin and Gallin, 1977; Ueda *et al.* 1975, 1985). Our experiments show that cells depolarized by azide or by electroporation exhibit normal chemotaxis in a cyclic AMP gradient. We conclude that membrane potential-regulated processes are not significantly involved in regulation of chemotaxis in *D. discoideum*. Hence, if the ion fluxes observed upon chemotactic stimulation (Bumann *et al.* 1984, 1986; Malchow *et al.* 1978, 1982; Böhme *et al.* 1987; Aeckerle *et al.* 1985; Aerts *et al.* 1987) are necessary for chemotaxis, these fluxes will not be under the control of the membrane potential. Since large non-ion-selective conductances are present in electroporated cells and intracellular Na^+ and K^+ concentrations are not strongly buffered in *D. discoideum* (Maeda, 1983), the data also suggest that K^+ and Na^+ fluxes are not important in the regulation of chemotaxis. The present experiments do not inform us about a possible role of calcium fluxes in chemotaxis, since the intracellular calcium concentration is strongly buffered and regulated, as in other eukaryotic cells. The use of electroporated cells in strongly buffered calcium solutions may permit a study of the role of intracellular calcium in chemotaxis.

Both azide- and electroporation-depolarized cells have a normal cyclic GMP response upon stimulation with cyclic AMP. Therefore, membrane potential-regulated processes are not involved in the cyclic GMP response. In contrast, the cyclic AMP-mediated cyclic AMP response is inhibited in both azide-treated and electroporated cells. This confirms the general finding (e.g. see Newell *et al.* 1987; McRobbie, 1986) that the intracellular cyclic AMP response is not involved in chemotaxis. The absence of cyclic AMP relay in electroporated and azide-treated cells is most likely not directly due to membrane potential depolarization. This depolarization can also be induced by incubating cells at low extracellular pH (Van Duijn and Vogelzang, 1989); however, this treatment does not inhibit cyclic AMP-induced cyclic AMP accumulation (P. Devreotes, personal communication). Dinauer and coworkers (1980) suggest that azide can block a step between binding of cyclic AMP to the plasma membrane receptor and activation of adenylate cyclase. A role for calcium in the activation of adenylate cyclase cannot be excluded.

Chemotaxis is a complex process that includes the detection of gradients of chemoattractant, the transduction of temporo-spatial information about the chemotactic gradient and the analysis of this information in terms of directed pseudopod formation. In this study we have shown that cells with depolarized plasma membrane

potential show a normal chemotactic response. These results suggest that voltage-dependent processes and fluxes of ions over the plasma membrane do not play an important role in chemotaxis.

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